

Supporting Information

Understanding the Sequence Preference of Recurrent RNA Building Blocks
using Quantum Chemistry:

The Intrastrand RNA Dinucleotide Platform

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Table S1 Crystal structures used to derive GpU, ApA, and UpC dinucleotide platforms. In two cases (1q9a and 483d), both alternative atom positions of the 5'-residue were used (labeled as A/B).

| Platform type | PDB ID | Residues:Chain | Label | X-ray structure resolution [Å] | Reference |
|---------------|--------|----------------|------------|--------------------------------|-----------|
| GpU | 1q9a | 2655-2656:A | GU-1q9a-1A | 1.04 | [S1] |
| | | 2655-2656:A | GU-1q9a-1B | | |
| | 483d | 2655-2656:A | GU-483d-1A | 1.11 | [S2] |
| | | 2655-2656:A | GU-483d-1B | | |
| | 480d | 2655-2656:A | GU-480d-1 | 1.50 | [S2] |
| | 1msy | 2655-2656:A | GU-1msy-1 | 1.41 | [S3] |
| | 3dw4 | 2655-2656:A | GU-3dw4-1 | 0.97 | [S4] |
| | 3dw6 | 2655-2656:A | GU-3dw6-1 | 1.00 | [S4] |
| | 3dvz | 2655-2656:A | GU-3dvz-1 | 1.00 | [S4] |
| | 1q96 | 10-11:A | GU-1q96-1 | 1.75 | [S1] |
| | | 10-11:B | GU-1q96-2 | | |
| | | 10-11:C | GU-1q96-3 | | |
| | 1q93 | 10-11:A | GU-1q93-1 | 2.25 | [S1] |
| | | 10-11:C | GU-1q93-2 | | |
| | 1y27 | 62-31:X | GU-1y27-1 | 2.40 | [S5] |
| | 2ees | 62-31:A | GU-2ees-1 | 1.75 | [S6] |
| | 1u8d | 62-31:A | GU-1u8d-1 | 1.95 | [S7] |
| | 2b57 | 62-31:A | GU-2b57-1 | 2.15 | [S8] |
| | 2g9c | 62-31:A | GU-2g9c-1 | 1.70 | [S9] |
| | 2qus | 20-21:A | GU-2qus-1 | 2.40 | [S10] |
| | 2quw | 20-21:A | GU-2quw-1 | 2.20 | [S10] |
| | 1jj2 | 1292-1293:0 | GU-1jj2-1 | 2.40 | [S11] |
| | | 175-176:0 | GU-1jj2-2 | | |
| | | 213-214:0 | GU-1jj2-3 | | |
| | | 358-359:0 | GU-1jj2-4 | | |
| | | 381-382:0 | GU-1jj2-5 | | |
| | | 464-465:0 | GU-1jj2-6 | | |
| | | 1370-1371:0 | GU-1jj2-7 | | |
| | | 1971-1972:0 | GU-1jj2-8 | | |
| | | 2692-2693:0 | GU-1jj2-9 | | |
| | | 78-79:9 | GU-1jj2-10 | | |
| | 3dil | 27-28:A | GU-3dil-1 | 1.90 | [S12] |
| ApA | 1gid | 218-219:A | AA-1gid-1 | 2.50 | [S13] |
| | | 218-219:B | AA-1gid-3 | | |
| | | 225-226:A | AA-1gid-2 | | |
| | | 225-226:B | AA-1gid-4 | | |
| | 1hr2 | 225-226:A | AA-1hr2-1 | 2.25 | [S14] |
| | | 225-226:B | AA-1hr2-2 | | |
| | | 171-172:A | AA-1hr2-3 | | |
| | | 171-172:B | AA-1hr2-4 | | |
| | | 218-219:B | AA-1hr2-5 | | |
| | 2r8s | 172-172:R | AA-2r8s-1 | 1.95 | [S15] |
| | | 225-226:R | AA-2r8s-2 | | |
| | 1jj2 | 1193-1194:0 | AA-1jj2-1 | 2.40 | [S11] |
| | | 441-442:0 | AA-1jj2-2 | | |
| | | 51-52:9 | AA-1jj2-3 | | |
| UpC | 1drz | 155-156:B | UC-1drz-1 | 2.30 | [S16] |
| | 1jj2 | 1009-1010:0 | UC-1jj2-1 | 2.40 | [S11] |

| | | | | | |
|--|------|-----------|-----------|------|-------|
| | 1sj3 | 155-156:R | UC-1sj3-1 | 2.20 | [S17] |
| | 1u0b | 73-74:A | UC-1u0b-1 | 2.30 | [S18] |
| | 1vc7 | 155-156:B | UC-1vc7-1 | 2.45 | [S17] |

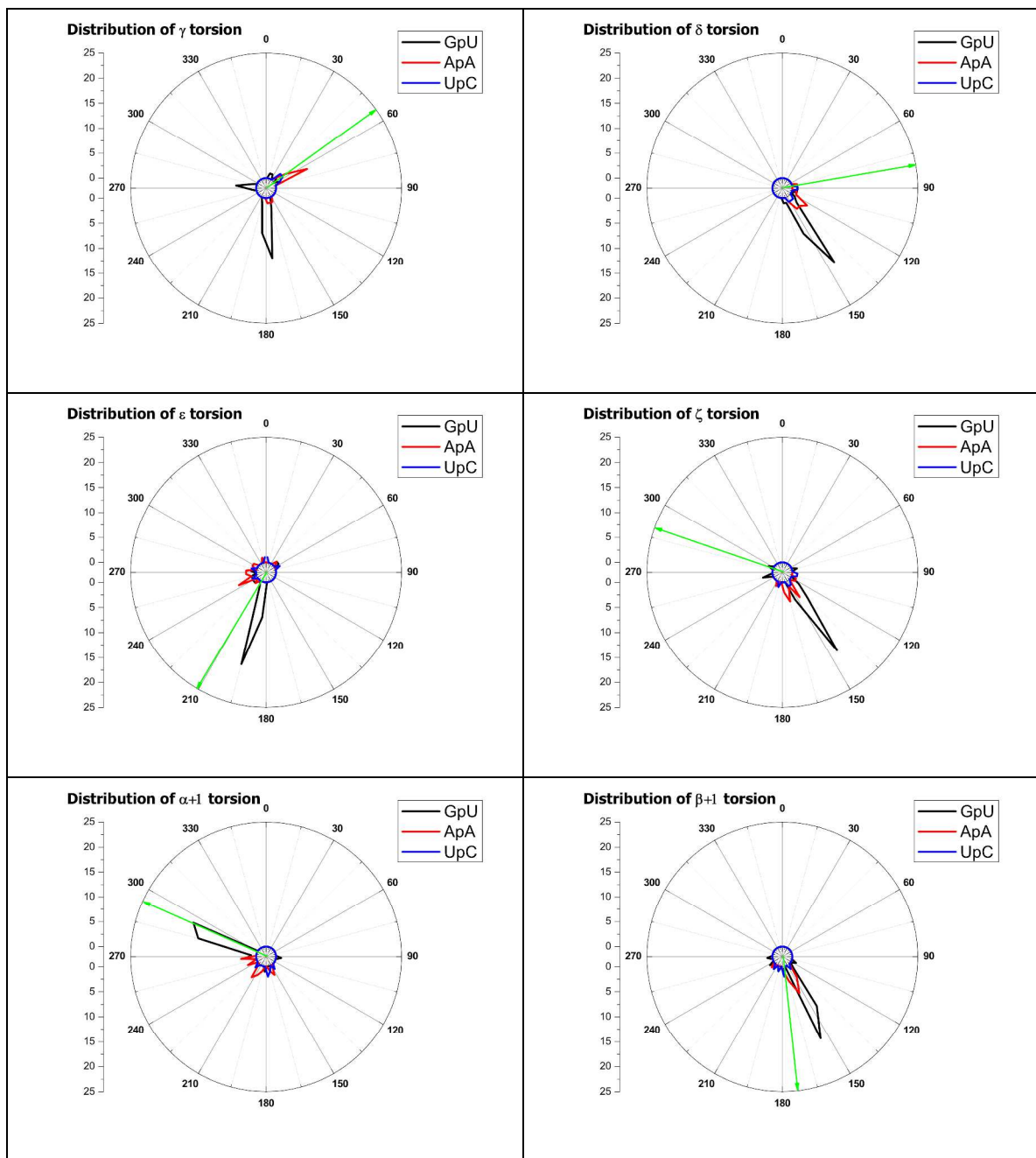
References

- [S1] Correll, C. C.; Beneken, J.; Plantinga, M. J.; Lubbers, M.; Chan, Y. L. *Nucleic Acids Res.* **2003**, *31*, 6806-6818
- [S2] Correll, C. C.; Wool, I. G.; Munishkin, A. *J. Mol. Biol.* **1999**, *292*, 275-287
- [S3] Correll, C. C.; Swinger, K. *RNA* **2003**, *9*, 355-363
- [S4] Olieric, V.; Rieder, U.; Lang, K.; Serganov, A.; Schulze-Briese, C.; Micura, R.; Dumas, P.; Ennifar, E. *RNA* **2009**, *15*, 707-715
- [S5] Serganov, A.; Yuan, Y. R.; Pikovskaya, O.; Polonskaia, A.; Malinina, L.; Phan, A. T.; Hobartner, C.; Micura, R.; Breaker, R. R.; Patel, D. J. *Chem. Biol.* **2004**, *11*, 1729-1741
- [S6] Gilbert, S. D.; Love, C. E.; Edwards, A. L.; Batey, R. T. *Biochemistry* **2007**, *46*, 13297-13309
- [S7] Batey, R. T.; Gilbert, S. D.; Montange, R. K. *Nature* **2004**, *432*, 411-415
- [S8] Gilbert, S. D.; Stoddard, C. D.; Wise, S. J.; Batey, R. T. *J. Mol. Biol.* **2006**, *359*, 754-768
- [S9] Gilbert, S. D.; Mediatore, S. J.; Batey, R. T. *J. Am. Chem. Soc.* **2006**, *128*, 14214-14215
- [S10] Chi, Y. I.; Martick, M.; Lares, M.; Kim, R.; Scott, W. G.; Kim, S. H. *PLoS Biol.* **2008**, *6*, 2060-2068
- [S11] Klein, D. J.; Schmeing, T. M.; Moore, P. B.; Steitz, T. A. *EMBO J.* **2001**, *20*, 4214-4221
- [S12] Serganov, A.; Huang, L.; Patel, D. J. *Nature* **2008**, *455*, 1263-1276
- [S13] Cate, J. H.; Gooding, A. R.; Podell, E.; Zhou, K.; Golden, B. L.; Kundrot, C. E.; Cech, T. R.; Doudna, J. A. *Science* **1996**, *273*, 1678-1685
- [S14] Juneau, K.; Podell, E.; Harrington, D. J.; Cech, T. R. *Structure* **2001**, *9*, 221-231
- [S15] Ye, J. D.; Tereshko, V.; Frederiksen, J. K.; Koide, A.; Fellouse, F. A.; Sidhu, S. S.; Koide, S.; Kossiakoff, A. A.; Piccirilli, J. A. *Proc. Natl. Acad. Sci. U. S. A* **2008**, *105*, 82-87
- [S16] Ferre-D'Amare, A. R.; Zhou, K.; Doudna, J. A. *Nature* **1998**, *395*, 567-574
- [S17] Ke, A.; Zhou, K.; Ding, F.; Cate, J. H.; Doudna, J. A. *Nature* **2004**, *429*, 201-205
- [S18] Hauenstein, S.; Zhang, C. M.; Hou, Y. M.; Perona, J. J. *Nat. Struct. Mol. Biol.* **2004**, *11*, 1134-1141

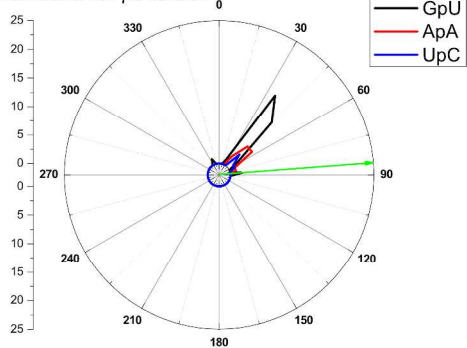
Table S2 Backbone and glycosidic torsion angles, in degrees, of the studied dinucleotide platforms supplemented with polar distribution plots. The green arrow in each graph denotes the average torsion of the A-RNA reference state.

| System | γ | δ | ϵ | ζ | $\alpha+1$ | $\beta+1$ | $\gamma+1$ | $\delta+1$ | χ | $\chi+1$ |
|------------|----------|----------|------------|---------|------------|-----------|------------|------------|--------|----------|
| GU-1q9a-1A | 179 | 147 | 192 | 142 | 291 | 152 | 39 | 89 | 263 | 183 |
| GU-1q9a-1B | 275 | 170 | 194 | 128 | 286 | 152 | 39 | 89 | 287 | 183 |
| GU-483d-1A | 178 | 147 | 194 | 142 | 288 | 152 | 42 | 90 | 261 | 183 |
| GU-483d-1B | 275 | 164 | 189 | 134 | 288 | 152 | 42 | 90 | 284 | 183 |
| GU-480d-1 | 267 | 158 | 190 | 140 | 295 | 146 | 37 | 88 | 277 | 181 |
| GU-1msy-1 | 179 | 149 | 193 | 141 | 287 | 158 | 38 | 92 | 266 | 186 |
| GU-3dw4-1 | 183 | 152 | 192 | 142 | 286 | 157 | 40 | 90 | 272 | 185 |
| GU-3dw6-1 | 184 | 151 | 193 | 141 | 285 | 157 | 40 | 90 | 274 | 184 |
| GU-3dvz-1 | 181 | 152 | 193 | 141 | 290 | 153 | 39 | 90 | 269 | 182 |
| GU-1q96-1 | 175 | 147 | 195 | 141 | 296 | 148 | 34 | 85 | 258 | 185 |
| GU-1q96-2 | 271 | 151 | 187 | 145 | 297 | 150 | 32 | 86 | 271 | 187 |
| GU-1q96-3 | 175 | 144 | 200 | 139 | 299 | 151 | 30 | 87 | 255 | 189 |
| GU-1q93-1 | 174 | 150 | 193 | 136 | 295 | 146 | 38 | 89 | 263 | 187 |
| GU-1q93-2 | 173 | 149 | 194 | 141 | 296 | 145 | 39 | 86 | 256 | 184 |
| GU-1y27-1 | 162 | 100 | 43 | 295 | 226 | 268 | 85 | 100 | 252 | 209 |
| GU-2ees-1 | 179 | 148 | 249 | 124 | 263 | 178 | 34 | 102 | 268 | 219 |
| GU-1u8d-1 | 190 | 139 | 235 | 142 | 286 | 153 | 27 | 90 | 267 | 209 |
| GU-2b57-1 | 183 | 137 | 251 | 146 | 264 | 154 | 32 | 83 | 268 | 204 |
| GU-2g9c-1 | 185 | 143 | 226 | 137 | 288 | 155 | 38 | 146 | 263 | 210 |
| GU-2qus-1 | 24 | 93 | 53 | 257 | 96 | 112 | 336 | 166 | 266 | 238 |
| GU-2quw-1 | 18 | 123 | 278 | 72 | 180 | 234 | 89 | 129 | 278 | 217 |
| GU-1jj2-1 | 63 | 88 | 183 | 256 | 288 | 194 | 50 | 87 | 194 | 186 |
| GU-1jj2-2 | 174 | 148 | 196 | 143 | 287 | 153 | 42 | 86 | 261 | 185 |
| GU-1jj2-3 | 182 | 148 | 185 | 154 | 293 | 149 | 42 | 83 | 264 | 183 |
| GU-1jj2-4 | 274 | 149 | 185 | 158 | 276 | 149 | 53 | 85 | 281 | 189 |
| GU-1jj2-5 | 280 | 156 | 190 | 140 | 290 | 137 | 46 | 82 | 275 | 185 |
| GU-1jj2-6 | 177 | 150 | 195 | 145 | 290 | 151 | 44 | 85 | 258 | 182 |
| GU-1jj2-7 | 164 | 149 | 192 | 145 | 291 | 149 | 41 | 83 | 261 | 188 |
| GU-1jj2-8 | 43 | 142 | 197 | 134 | 288 | 145 | 39 | 84 | 276 | 189 |
| GU-1jj2-9 | 179 | 148 | 189 | 150 | 294 | 141 | 41 | 82 | 255 | 185 |
| GU-1jj2-10 | 178 | 148 | 185 | 150 | 291 | 149 | 44 | 85 | 268 | 185 |
| GU-3dil-1 | 181 | 141 | 196 | 149 | 288 | 153 | 39 | 85 | 262 | 187 |
| AA-1gid-1 | 64 | 140 | 243 | 168 | 260 | 144 | 39 | 86 | 233 | 179 |
| AA-1gid-3 | 60 | 138 | 243 | 168 | 262 | 144 | 38 | 86 | 233 | 178 |
| AA-1gid-2 | 50 | 123 | 241 | 168 | 245 | 150 | 47 | 89 | 220 | 184 |
| AA-1gid-4 | 50 | 122 | 244 | 168 | 243 | 151 | 47 | 89 | 222 | 187 |
| AA-1hr2-1 | 48 | 133 | 275 | 149 | 212 | 155 | 50 | 86 | 249 | 187 |
| AA-1hr2-2 | 60 | 115 | 291 | 145 | 200 | 154 | 55 | 87 | 226 | 189 |
| AA-1hr2-3 | 156 | 129 | 308 | 172 | 196 | 166 | 43 | 85 | 194 | 181 |
| AA-1hr2-4 | 64 | 109 | 345 | 129 | 165 | 175 | 55 | 86 | 206 | 186 |
| AA-1hr2-5 | 177 | 79 | 49 | 112 | 159 | 164 | 61 | 85 | 175 | 195 |
| AA-2r8s-1 | 60 | 137 | 265 | 148 | 212 | 153 | 57 | 84 | 237 | 185 |
| AA-2r8s-2 | 60 | 125 | 278 | 144 | 215 | 155 | 47 | 81 | 232 | 189 |
| AA-1jj2-1 | 58 | 149 | 262 | 206 | 205 | 167 | 54 | 83 | 210 | 180 |
| AA-1jj2-2 | 61 | 149 | 225 | 179 | 263 | 147 | 43 | 82 | 234 | 188 |
| AA-1jj2-3 | 165 | 156 | 257 | 153 | 157 | 225 | 75 | 82 | 219 | 197 |
| UC-1drz-1 | 55 | 130 | 354 | 149 | 174 | 149 | 43 | 80 | 240 | 188 |
| UC-1jj2-1 | 66 | 151 | 249 | 196 | 221 | 172 | 45 | 77 | 231 | 203 |

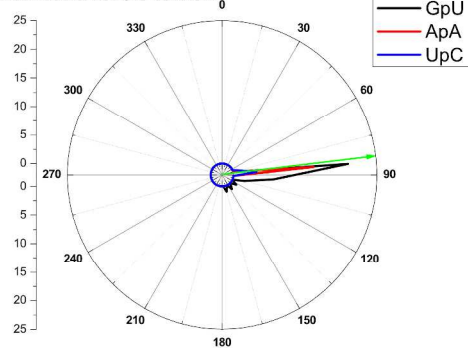
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|-----------|----|-----|-----|-----|-----|-----|----|----|-----|-----|
| UC-1sj3-1 | 48 | 144 | 280 | 150 | 162 | 216 | 68 | 87 | 243 | 196 |
| UC-1u0b-1 | 56 | 84 | 62 | 105 | 147 | 173 | 56 | 81 | 188 | 214 |
| UC-1vc7-1 | 47 | 92 | 7 | 95 | 172 | 191 | 49 | 81 | 215 | 183 |



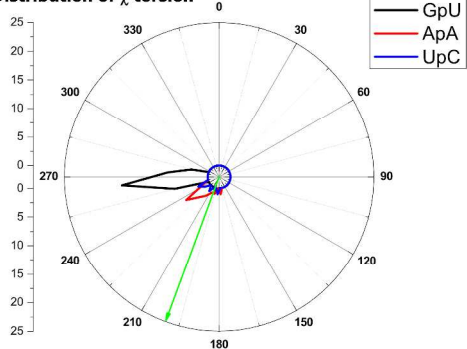
Distribution of $\gamma+1$ torsion₀



Distribution of $\delta+1$ torsion₀



Distribution of χ torsion



Distribution of $\chi+1$ torsion₀

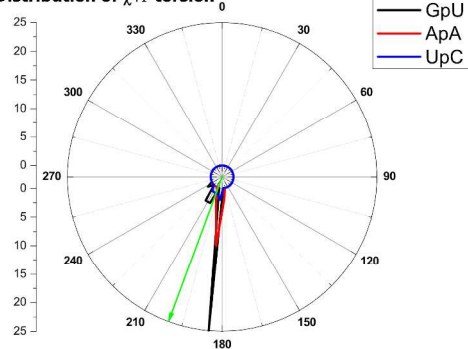


Table S3 DF-MP2/aug-cc-pVDZ CP-corrected (i.e. BSSE-free) interaction energies of GU, AA, and UC base pairs. The pairs were derived from the TPSS-D/LP optimized platform dinucleotides. The appended methyl groups were relaxed using M06/6-31+G(d, p) prior to computation of the interaction energies.

| Base pair | System | ΔE [kcal.mol ⁻¹] |
|-----------|------------|--------------------------------------|
| GU | GU-1q9a-1A | -8.1 |
| | GU-1q9a-1B | -8.2 |
| | GU-483d-1A | -8.1 |
| | GU-483d-1B | -8.3 |
| | GU-480d-1 | -8.2 |
| | GU-1msy-1 | -8.1 |
| | GU-3dw4-1 | -8.0 |
| | GU-3dw6-1 | -8.0 |
| | GU-3dvz-1 | -8.3 |
| | GU-1q96-1 | -8.1 |
| | GU-1q96-2 | -8.1 |
| | GU-1q96-3 | -7.5 |
| | GU-1q93-1 | -7.6 |
| | GU-1q93-2 | -8.1 |
| | GU-1y27-1 | -8.8 |
| | GU-2ees-1 | -6.5 |
| | GU-1u8d-1 | -6.1 |
| | GU-2b57-1 | -7.8 |
| | GU-2g9c-1 | -8.5 |
| | GU-2qus-1 | -8.9 |
| | GU-2quw-1 | -8.2 |
| | GU-1jj2-1 | -8.1 |
| | GU-1jj2-2 | -8.1 |
| | GU-1jj2-3 | -8.1 |
| | GU-1jj2-4 | -7.9 |
| | GU-1jj2-5 | -8.1 |
| | GU-1jj2-6 | -8.1 |
| | GU-1jj2-7 | -8.0 |
| | GU-1jj2-8 | -7.7 |
| | GU-1jj2-9 | -8.0 |
| | GU-1jj2-10 | -7.9 |
| | GU-3dil-1 | -7.7 |
| AA | AA-1gid-1 | -6.5 |
| | AA-1gid-3 | -6.5 |
| | AA-1gid-2 | -7.1 |
| | AA-1gid-4 | -7.1 |
| | AA-1hr2-1 | -6.6 |
| | AA-1hr2-2 | -7.1 |
| | AA-1hr2-3 | -7.3 |
| | AA-1hr2-4 | -6.9 |
| | AA-1hr2-5 | -2.6 |
| | AA-2r8s-1 | -6.3 |
| | AA-2r8s-2 | -6.4 |
| | AA-1jj2-1 | -6.9 |
| | AA-1jj2-2 | -6.4 |
| | AA-1jj2-3 | -7.1 |
| UC | UC-1drz-1 | -5.6 |

| | | |
|--|-----------|------|
| | UC-1jj2-1 | -5.6 |
| | UC-1sj3-1 | -5.5 |
| | UC-1u0b-1 | -4.8 |
| | UC-1vc7-1 | -4.9 |

Table S4 RI-MP2/CBS relative energies of rSPSOM models of GpU, ApA, and UpC dinucleotide platforms. The energies are expressed as relative values with respect to the rSPSOM model of the A-RNA reference state.

| rSPSOM type | System | E [kcal.mol ⁻¹] |
|-------------|------------|-----------------------------|
| GpU | GU-1q9a-1A | −0.8 |
| | GU-1q9a-1B | +5.0 |
| | GU-483d-1A | −0.9 |
| | GU-483d-1B | +4.7 |
| | GU-480d-1 | +1.2 |
| | GU-1msy-1 | −0.9 |
| | GU-3dw4-1 | −0.6 |
| | GU-3dw6-1 | −0.3 |
| | GU-3dvz-1 | −0.3 |
| | GU-1q96-1 | −0.8 |
| | GU-1q96-2 | +0.4 |
| | GU-1q96-3 | −1.2 |
| | GU-1q93-1 | +0.5 |
| | GU-1q93-2 | −0.7 |
| | GU-1y27-1 | +1.4 |
| | GU-2ees-1 | +7.0 |
| | GU-1u8d-1 | −0.2 |
| | GU-2b57-1 | +0.4 |
| | GU-2g9c-1 | −0.4 |
| | GU-2qus-1 | +4.6 |
| | GU-2quw-1 | +4.0 |
| | GU-1jj2-1 | −3.6 |
| | GU-1jj2-2 | −1.5 |
| | GU-1jj2-3 | −1.2 |
| | GU-1jj2-4 | −0.4 |
| | GU-1jj2-5 | +0.6 |
| | GU-1jj2-6 | −1.4 |
| | GU-1jj2-7 | −1.2 |
| | GU-1jj2-8 | −0.3 |
| | GU-1jj2-9 | −1.2 |
| | GU-1jj2-10 | −0.6 |
| | GU-3dil-1 | −2.2 |
| ApA | AA-1gid-1 | +0.9 |
| | AA-1gid-3 | +0.4 |
| | AA-1gid-2 | +1.2 |
| | AA-1gid-4 | +1.1 |
| | AA-1hr2-1 | +0.4 |
| | AA-1hr2-2 | +2.4 |
| | AA-1hr2-3 | +6.2 |
| | AA-1hr2-4 | +6.1 |
| | AA-1hr2-5 | +3.9 |
| | AA-2r8s-1 | +2.1 |
| | AA-2r8s-2 | −0.1 |
| | AA-1jj2-1 | +1.3 |
| | AA-1jj2-2 | −1.1 |
| | AA-1jj2-3 | +2.8 |
| UpC | UC-1drz-1 | +6.1 |
| | UC-1jj2-1 | +2.1 |

| | | |
|--|-----------|------|
| | UC-1sj3-1 | -3.1 |
| | UC-1u0b-1 | +0.9 |
| | UC-1vc7-1 | -0.9 |

Table S5 Grouping of GpU, ApA, and UpC rSPSOM platform models based on the conformational classification software of Richardson *et al.* Models that could not be assigned a conformational type are marked as unknown (U). Roman numerals denote the categories discussed in the text and symbols in parentheses are those suggested by Richardson *et al.* and reported in the computer output. The suiteness value between 0 and 1 denotes the similarity of the rSPSOM to the average structure in the particular class. The A/B suffixes denoting particular conformational substates of the bi-conformational structures are highlighted in boldface.

| rSPSOM type | System | Conformational class | Suiteness |
|-------------|--------------------|----------------------|-----------|
| GpU | GU-1q9a-1 A | II (#a) | 0.926 |
| | GU-1q9a-1 B | II (#a) | 0.103 |
| | GU-483d-1 A | II (#a) | 0.923 |
| | GU-483d-1 B | II (#a) | 0.379 |
| | GU-480d-1 | II (#a) | 0.667 |
| | GU-1msy-1 | II (#a) | 0.842 |
| | GU-3dw4-1 | II (#a) | 0.894 |
| | GU-3dw6-1 | II (#a) | 0.904 |
| | GU-3dvz-1 | II (#a) | 0.896 |
| | GU-1q96-1 | II (#a) | 0.847 |
| | GU-1q96-2 | II (#a) | 0.768 |
| | GU-1q96-3 | II (#a) | 0.671 |
| | GU-1q93-1 | II (#a) | 0.862 |
| | GU-1q93-2 | II (#a) | 0.922 |
| | GU-1y27-1 | U (!!) | - |
| | GU-2ees-1 | III (0a) | 0.095 |
| | GU-1u8d-1 | III (0a) | 0.279 |
| | GU-2b57-1 | III (0a) | 0.213 |
| | GU-2g9c-1 | IV (4g) | 0.431 |
| | GU-2qus-1 | U (!!) | - |
| | GU-2quw-1 | U (!!) | - |
| | GU-1jj2-1 | I (&a) | 0.785 |
| | GU-1jj2-2 | II (#a) | 0.977 |
| | GU-1jj2-3 | II (#a) | 0.928 |
| | GU-1jj2-4 | II (#a) | 0.681 |
| | GU-1jj2-5 | II (#a) | 0.774 |
| | GU-1jj2-6 | II (#a) | 0.989 |
| | GU-1jj2-7 | II (#a) | 0.982 |
| | GU-1jj2-8 | II (#a) | 0.803 |
| | GU-1jj2-9 | II (#a) | 0.927 |
| | GU-1jj2-10 | II (#a) | 0.959 |
| | GU-3dil-1 | II (#a) | 0.832 |
| ApA | AA-1gid-1 | III (0a) | 0.196 |
| | AA-1gid-3 | III (0a) | 0.184 |
| | AA-1gid-2 | U (!!) | - |
| | AA-1gid-4 | U (!!) | - |
| | AA-1hr2-1 | IV (4g) | 0.348 |
| | AA-1hr2-2 | U (!!) | - |
| | AA-1hr2-3 | IV (4g) | 0.010 |
| | AA-1hr2-4 | U (!!) | - |
| | AA-1hr2-5 | U (!!) | - |
| | AA-2r8s-1 | IV (4g) | 0.520 |
| | AA-2r8s-2 | IV (4g) | 0.039 |
| | AA-1jj2-1 | IV (4g) | 0.146 |
| | AA-1jj2-2 | III (0a) | 0.124 |

| | | | |
|-----|-----------|---------|-------|
| | AA-1jj2-3 | U (!!) | - |
| UpC | UC-1drz-1 | U (!!) | - |
| | UC-1jj2-1 | IV (4g) | 0.339 |
| | UC-1sj3-1 | U (!!) | - |
| | UC-1u0b-1 | U (!!) | - |
| | UC-1vc7-1 | U (!!) | - |

A-RNA optimization constraints

Optimization constraints were imposed on the quasi- β and quasi- $\epsilon+1$ torsions of the A-RNA backbone based on the following rationale: (i) given that the A-RNA conformational substate is periodic, corresponding torsions in different nucleotide units are assumed to adopt the same values (ignoring any sequence preferences), and (ii) if the quasi- β torsion is free to relax, the 5'-terminal methoxy group is pulled toward the phosphate group via a $C_{Met}\cdots H\cdots O1P$ interaction, with the resultant $C_{Met}\cdots O1P$ distance of ~ 3.5 Å leading to a non-realistic stabilization of the structure. Such extensive bending of the backbone is biologically irrelevant as it would lead to a strong electrostatic repulsion between the negatively charged phosphates. In line with this, no such backbone deviation has been observed in the experimentally available geometries. For similar reasons, the quasi- $\epsilon+1$ dihedral was fixed as well at the A-RNA average value. Note that no such backbone flexion was observed for the platform systems throughout geometry minimization and thus both quasi- β and quasi- $\epsilon+1$ dihedrals were optimized.

Clarification of exclusion the AA-1hr2-5 base pair from statistics

The lower stability of the AA-1hr2-5 system is probably due to the inaccurately resolved experimental geometry of the sugar-phosphate backbone as the 3'-nucleotide is shifted in such a way that the C2 of the 5'-adenine and the N6 of the 3'-adenine are positioned as close as 2.7 Å. Despite extensive constraints imposed on the backbone and the two glycosidic torsion angles, the experimentally predicted coplanarity and the pairing pattern of the nucleobases are disrupted upon optimization.

GpU rSPSOM Outliers - In-depth Analysis

The **A/B** suffixes distinguishing conformational substates of the bi-conformational structures, i.e. GU-1q9a-1 and GU-483d-1, are highlighted in boldface.

- **Platforms derived from high-resolution structures:** 1q9a and 483d

Two rSPSOMs were derived from each of the 1q9a and 483d structures (high resolution X-ray structures of the *Escherichia coli* sarcin/ricin domain which included refinement of two 5'-residue geometries), here named GU-1q9a-1**A/B** and GU-483d-1**A/B**, where **A/B** refers to one of the two alternative conformations of the 5'-residue (G). Both **B**-labeled substates show noticeably lower intrinsic stabilities compared to the **A** substates. This difference correlates well with the low *S*-values of the **B**-alternatives (*S*-values of **A/B** conformational variants being 0.93/0.10 and 0.92/0.38 for GU-1q9a-**A/B** and GU-483d-**A/B**, respectively). The rSPSOM model of the GU-1q9a-1**B** backbone, which is 5.8 kcal.mol⁻¹ higher in energy than that of the **A** variant (and 5.0 kcal.mol⁻¹ above that of the A-RNA reference), contains a steric clash between H2'...H5' (2.1 Å) brought about by high δ (170°) and a *gauche*⁻ state of γ . The uncompensated shift of ζ , from 142° (**A** variant) to 128° (**B** variant), in combination with high δ , probably weakens the O2'...O2P interaction. Given that the **A/B** alternatives only differ in the sugar-phosphate backbone of the 5'-residue, the structural variations of GU-1q9a-1**B** might represent a destabilization of the system. The rationale for the lower stability of the rSPSOM model of GU-483d-1**B**, with energy 5.6 kcal.mol⁻¹ above the **A**-alternative (and 4.7 kcal.mol⁻¹ above A-RNA), is similar to that for the GU-1q9a-1**A/B** pair, i.e., high δ (164°), a slight H2'...H5' steric clash (2.2 Å), and ϵ/ζ alteration diminishing the stabilizing effect of the O2'...O2P H-bond.

- **Platforms derived from medium/low-resolution structures:** 2ees, 2qus, 2quw

The lower stability of the conformationally uncharacterized GU-2qus-1 (U) system is partially due to a clash of H4'...H5'(n+1) (1.9 Å) caused by the unusual setting of several backbone torsions. In the case of GU-2quw-1 (U), the electrostatic repulsion between the O5'(n+1) and O4'(n+1) lone pairs leads to a pucker transition from C2'-endo to O4'-exo. Although the O5'(n+1)...O4'(n+1) distance remains the same, the latter pucker directs the upper lone pair (the one on the 5'-side of the sugar ring) away from that of O5'. The energetically least stable structure with relative energy 7.0 kcal.mol⁻¹ above A-RNA is GU-2ees-1 (with class/suiteness values of III/0.10). Clarification of the energetic penalty here is not straightforward as there are a number of different factors, which in concert render the conformer less stable. This statement

also holds for the preceding high-energy systems even though several possible reasons for inferior stabilities (e.g., steric clashes, suboptimal structural parameters of O2'...O2P H-bonds, etc.) have been suggested. Note that the interconnection of the strongly correlated backbone torsion angles often precludes identification of the source of the high energy in the system. Also note that the five outliers of lower intrinsic stability GU(-1q9a-1B, -483d-1B, -2qus-1, -2quw-1, -2ees-1) either could not be unambiguously assigned to any of the 46 established RNA backbone conformational classes or were assigned to a known class with a low value of confidence, i.e., suiteness. This finding suggests that the initial X-ray structures of these five conformers are rather atypical and might have been resolved with insufficient resolution (likely in the case of GU-2qus-1, GU-2quw-1, GU-2ees-1) or might represent unstable backbone substates (probably in the case of GU-1q9a-1B, GU-483d-1B), which might be compensated by stabilizing interactions elsewhere in the motif.

ApA rSPSOM Outliers - In-depth Analysis

We have tried to analyze the individual structures and to identify some sources of the variability in energy. The magnitude of the standard deviation in the relative energies is predominantly influenced by the AA-1hr2-3 and AA-1hr2-4 systems, both of which are ~ 6 kcal.mol⁻¹ above the A-RNA reference structure. The markedly lower stability of AA-1hr2-3 (IV/0.01) is probably due to the occurrence of the ϵ torsion angle (308°) in the *gauche*⁻ domain, which is in a forbidden RNA region [S19, S20]. In the case of the unassigned AA-1hr2-4 (U) system, the ϵ torsion is shifted to even higher values (345°) not observed in experimental RNA structures [S19, S20]. The unnaturally high values of the ϵ torsion angles lead, among other things, to a steric clash between O1P and H4', which come as close as 2.2 Å and 2.1 Å in AA-1hr2-3 and AA-1hr2-4, respectively. The initial O1P...H4' distance in AA-1hr2-4 of ~ 1.6 Å induces a strain which (in combination with the constrained δ torsion) is relaxed during optimization via transition to an atypical C1'-endo pucker. The lower stability of the conformationally uncharacterized AA-1hr2-5 (U) system (3.9 kcal.mol⁻¹) could be also partially ascribed to the uncommon value of ϵ (49°), which initially positions H4' in proximity with O1P (1.8 Å). Although the H4'...O1P distance is extended throughout constrained optimization to 2.3 Å, some residual steric clash-related energetic penalty likely remains. The repulsion might be balanced, at least to a certain degree, by an unusual stabilizing O2'...O1P H-bond.

UpC rSPSOM - In-depth Analysis

The lower intrinsic stability of the UC-1drz-1 (U) structure is probably due to the high value of the ϵ torsion angle (354°), a state rarely observed in high-resolution experimental structures. Note that an ϵ torsion close to 0° positions the anionic O1P oxygen immediately below the 5'-sugar ring and likely leads to: (i) steric conflicts, e.g., the initial 1.8 Å distance of H4'...O1P in UC-1drz-1 increases in the course of our heavily constrained optimization to 2.1 Å, and (ii) electrostatic repulsion between O1P and O4'. Even though the starting, i.e., experimental, O1P...O4' distance in the UC-1drz-1 structure increases during minimization from 3.1 Å to 3.5 Å (within the limits imposed by extensive constraints), some residual tension likely remains. The O1P...O4' distance extension is also accompanied by a C3'-exo \rightarrow C1'-exo transition. The very low value of the ϵ torsion angle (7°) in UC-1vc7-1 (U), however, is compensated by the favorable setting of adjacent backbone dihedrals, which prevent the repulsive interactions observed in UC-1drz-1. Despite the unnatural value of the ϵ torsion (62°) in UC-1u0b-1 (a state incompatible with any of the RNA conformational classes), the structure is partially stabilized by an "outlandish" O2'...O1P H-bond (as in the case of the AA-1hr2-5 rSPSOM). The advantageous setting of the backbone dihedrals in the rSPSOM representation of the UC-1sj3-1 (U) system cooperatively renders this conformer to be of great stability. The fact that the given backbone conformation does not fit into any defined conformational class, however, raises questions about its suitability within a real physiological environment.

- [S19] Richardson, J. S.; Schneider, B.; Murray, L. W.; Kapral, G. J.; Immormino, R. M.; Headd, J. J.; Richardson, D. C.; Ham, D.; HersHKovits, E.; Williams, L. D.; Keating, K. S.; Pyle, A. M.; Micallef, D.; Westbrook, J.; Berman, H. M. *RNA*. **2008**, *14*, 465-481
- [S20] Schneider, B.; Moravek, Z.; Berman, H. M. *Nucleic Acids Res.* **2004**, *32*, 1666-1677

Molecular graphs of GU/AA/UC base pairs Molecular graphs of the most stable GU/AA/UC platform-derived base pairs obtained from the AIM analysis and showing all identified (3, -1) critical points (small red circles) indicative of interatomic interactions and (3, +1) critical points (yellow circles), which give evidence of a circle. The coloring of the atoms follows the standard convention, i.e., oxygen-red, nitrogen-blue, carbon-black, and hydrogen-gray.

